Pubertal induction in adolescents with DMD is associated with high satisfaction, gonadotropin release and increased muscle contractile surface area

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Abstract

Background

Pharmacological doses of glucocorticoids (GC) reduce inflammation and preserve muscle function in boys with Duchenne muscular dystrophy (DMD). Delayed puberty and bone fragility are consequences of GC treatment. The aim of this study was to determine the acceptability of a 2-year pubertal induction regimen using 4-weekly testosterone injections and examine changes in physique, bone integrity, muscle pathology (assessed by magnetic resonance imaging) and muscle function.

Methods

15 prepubertal males with DMD, aged 12-17 years and receiving GC, were treated with an incremental testosterone regimen for 2 years. Participants completed a Treatment Satisfaction Questionnaire (TSQM). Data on BMI, bone density, muscle pathology and function were collected at baseline and 2 years later.

Results

Testosterone injections were well tolerated, with high TSQM scores. Baseline BMI z-score was 2.16 (0.90) and 1.64 (1.35) 2 years later. Median testosterone levels were 9.7nmol/l (IQR 5.7-11.1) 6 – 9 months after the last injection with an associated increase in testicular volume. Lumbar spine z-score was 0.22 (SD 2.21) at baseline and 0.35 (SD 2.21) after 2 years. Upper and lower limb muscle contractile cross sectional area increased in all participants during the trial ($p=0.05$ and $p<0.01$ respectively). There was a reduction in T2 relaxation times in most muscle groups with stable upper limb muscle function.

Conclusion

Incremental monthly testosterone injections were well tolerated, promoted endogenous testosterone production and had a positive impact on the skeleton and contractile muscle bulk with evidence suggesting a beneficial impact on the underlying disease process.

Introduction

Duchenne muscular dystrophy (DMD) is a life-limiting genetic disorder that affects 1 in approximately 4000 newborn boys in the UK and occurs due to mutations in the *DMD* gene (1). Absence of the gene product, dystrophin, leads to increased muscle cell fragility and a cycle of degeneration and repair with inflammatory change and replacement of muscle fibres with fibrosis and fat (2). Pharmacological doses of glucocorticoids (GC) – either prednisolone or deflazacort – have had a beneficial impact on longevity and quality of life in DMD (3). Glucocorticoids stabilise muscle strength and have positive effects on cardiovascular and respiratory function (4) and help to reduce scoliosis (5). Unfortunately long-term high dose GC therapy has side-effects that include slow growth, weight gain, osteopaenia, secondary hypoadrenalism (6) and effects on mood (7). GC also prevent the normal progression into puberty, which can have significant psychological consequences. Exogenous androgen has been used for many years in boys with pubertal delay and is increasingly being used in adolescents with DMD (8). Exogenous androgen can have an anabolic effect on a range of different tissues and could mitigate some of the deleterious effects of GC on muscle, bone and psychological well-being. The increased life expectancy and changing expectations of adolescents with DMD underlines the importance of effective DMD management at this time. As yet, no one has examined the psychological, developmental and skeletal effects of testosterone in a prospective manner with a detailed examination of bone and muscle phenotype. A retrospective audit identified 14 boys who had been treated with testosterone for induction of puberty and highlighted the lack of consensus in terms of regimen, length of treatment or age at initiation (8). This study investigated the efficacy and tolerability of a 2-year regimen of incremental, intramuscular testosterone to induce puberty in a cohort of GC-treated adolescents with DMD.

Methods

Participants

Participants were from a single centre study that followed 15 adolescents with DMD and delayed puberty as they were treated with testosterone. Full details of the clinical trial and protocol have been published (9). Briefly, 15 prepubertal males between 12 and 17 years old were treated with a stepwise regimen of testosterone injections every 4 weeks. Data were collected to determine the effectiveness and tolerability of the treatment regimen. Inclusion and exclusion criteria are in the Supplementary Methods section.

Outcomes

Primary outcome

The primary outcome measure was Treatment Satisfaction Questionnaire for Medication (TSQM) (10).

Secondary outcomes

The secondary outcome measures were:

- Auxological assessment including height, weight and pubertal status (11)
- Biochemical assessment of pubertal stage
- Bone age
- Muscle cross-sectional area (CSA), contractile cross-sectional area (cCSA) and fat fraction (FF) determined by muscle MRI of upper and lower limbs, and T2 relaxation time of the upper limb
- Motor performance evaluated using North Star Ambulatory Assessment (NSAA) and Performance of Upper Limb (PUL)

- Bone mineral adjusted density of the lumbar spine and total body (minus head) using Dual-energy x-ray absorptiometry (DXA)

Testosterone regimen

The incremental testosterone regimen involved the administration of testosterone (Sustanon 250) by deep intramuscular injection over a 2-year period:

- Sustanon 50 mg (0.2 ml) every 4 weeks for 12 weeks
- Sustanon 100 mg (0.4 ml) every 4 weeks for 40 weeks
- Sustanon 150 mg (0.6 ml) every 4 weeks for 24 weeks
- Sustanon 250 mg (1 ml) every 4 weeks for 28 weeks

Assessment

Patient satisfaction

The TSQM is self-administered and used at baseline and 6 monthly thereafter. It is a 14-item instrument, yielding four subscale scores: global satisfaction, effectiveness, adverse events and convenience and has been validated for adults with chronic disease (10). The total score (excluding side-effects domain as these are only answered if the participant felt there were associated side-effects) was the primary outcome measure.

Clinical assessment

Height/arm span and weight were measured. Target height was calculated using: (mother + father's height)/2 +13cm. Testicular size was assessed using a Prader orchidometer and pubertal staging determined using Tanner Staging criteria (11). Assessments were conducted by CW or TC and cross-checked > 75% patients.

Biochemical gonadal / androgen status

Biochemical pubertal status was determined by measuring morning (pre-10.00) serum testosterone, luteinising hormone (LH) and follicle-stimulating hormone (FSH) concentrations at 0,6,12,18,24,27 and 30-33 months. Levels were also measured between 6 and 9 months after their last testosterone injection, as part of routine clinical follow-up.

Bone age

Bone age was assessed at baseline and 2 years using a radiograph of the left wrist and reported by a consultant radiologist (12).

Motor function and muscle strength

All participants performed PUL (Version 2.0) for assessing upper limb performance (13) and ambulatory patients also undertook the NSAA (14).

Bone mineral density

DXA scans were conducted annually using a Lunar iDXA (GE Lunar Corp, Madison, WI USA) to assess lean versus total body mass and enable calculation of bone mineral content (BMC), adjusted for age, size and gender. Adjusted z-scores of lumbar spine (L1-4) and total body (minus head) were recorded.

MRI data acquisition and image processing

Imaging was performed on a 3.0T scanner (detailed protocols in Supplementary Methods). Briefly, quantitative Dixon FF imaging was collected using fivefold compressed sensing acceleration (15, 16). For the upper limb, quantitative Dixon FF imaging and T2 relaxation time imaging (a surrogate of muscle inflammation (17)) was performed and maps processed. Regions of interest (ROIs) were defined for the upper and lower limb for multiple image slices and FF and T2 relaxation times were calculated together as area-weighted averages. CSA and cCSA were also calculated to quantify the area of viable muscle tissue remaining

(18). Results are presented for muscle groups across all participants, and for individual participants across all their muscle groups.

All participants consented to an MRI scan at baseline, 1 year and 2 years. Six healthy male controls, matched by bone age (as all participants in the trial had delayed puberty and bone age), underwent the same MRI protocol.

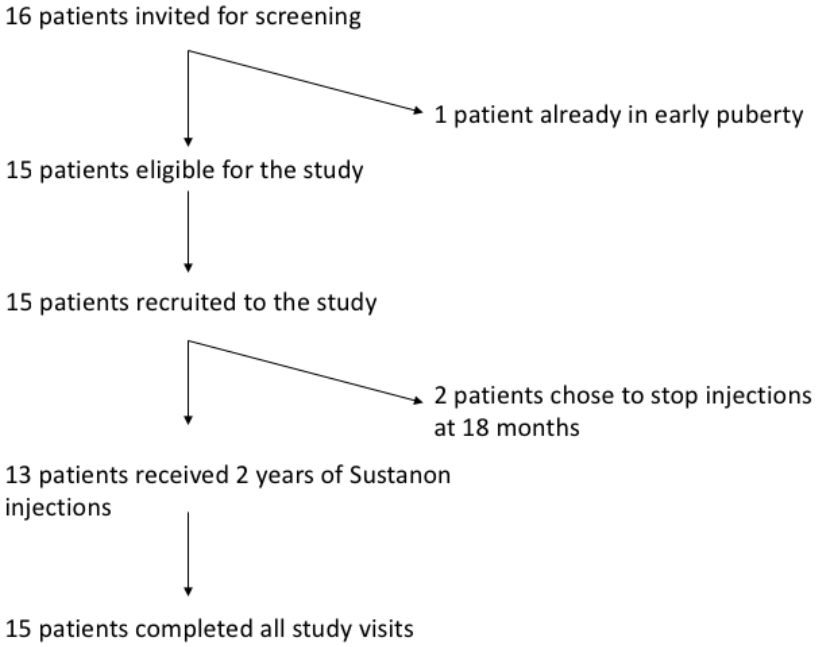
Statistical Analysis and approvals

This was an observational study and the primary outcome (treatment satisfaction) was not subject to a power calculation. The recruitment target was pragmatic and reflected the local population of boys with DMD and pubertal delay who were not already enrolled in a clinical trial. All data are presented as mean± SD unless stated otherwise. Independent t-tests were used to compare baseline measurements of DMD boys to healthy control participants. Paired t-tests (or Wilcoxon rank-sum techniques if variables not normally distributed) were used to compare outcome measures before and after testosterone administration. Statistical significance was taken to be $p<0.05$. Stata v15 was used for statistical analysis.

The study was approved by York Research Ethics Committee. Informed consent was obtained from the patient if $\geq$16 years and from the parent/guardian with patient assent if $\leq$16 years.

Results

Study flowchart



Baseline characteristics

Recruitment commenced in December 2015 until November 2016. 17 patients were approached, 16 agreed to take part and were screened and 15 were eligible (see flowchart and protocol (9)). The last participant's final visit was February 2019. Participants were aged 12.0-16.9 years (mean 13.8 years, mean bone age 9.4 years) at baseline (Table I). Controls were matched to bone age; their chronological age ranged from 7.9-12.9 years (mean 10.0 years), all controls were prepubertal. 3/15 participants were non-ambulant at baseline. All participants had been on GC therapy for a mean of 8.1 years at recruitment (Table II); regimens were unchanged during the study.

Two participants (15 and 16) in the study were happy with their pubertal development after 18 months of intramuscular testosterone and stopped injections but completed all scheduled visits. These are highlighted with * in figures and tables.

Treatment satisfaction for medication questionnaire

The mean total TSQM was 48.5 (SD 6.2, range 37-59) out of a possible 59 points, with similar scores in each domain (Table III and Figure I). To the nearest integer, the mean score for all but one treatment domain was 6 ('very satisfied'). When considering time to effect, the mean score was 5 ('satisfied'). Two patients felt that they had experienced side-effects from the IM testosterone regimen. Their responses corresponded with the adverse events profile recorded by the study team (Supplementary Results).

Pubertal status

All patients were either Tanner stage G1P1, G2P1 or G1P2 and had a testicular volume of <4mls at baseline. Initial testosterone levels were < 2.0nmol/l with suppressed LH and FSH levels (median LH <0.1IU/L (IQR <0.1, 0.1), median FSH 1.2IU/L (IQR 1.1, 1.8)). By the 2-year visit, patients were Tanner stage G4P4 to G5P5 and median testosterone level was 8.3nmol/l (IQR 5.7-17.9) 3-5 weeks after their last injection. Testicular volume was ≤ 5mls in all (mean 2.8, SD 2.9, range 1.5-5 mls) after 2 years and LH/FSH levels remained suppressed (median LH <0.1IU/L (IQR 0.1, 0.1), median FSH <0.1IU/L (IQR 0.1, 1.2)). By 3 months after the last Sustanon injection, activation of the hypothalamo-pituitary axis (HPA) was evident with a significant increase in both LH and FSH (median LH 6.7IU/L (IQR 5.9, 8.8), $p<0.01$, median FSH 6.0IU/L (IQR 4.4, 8.0), $p<0.01$) compared to baseline. In keeping with this, the median testosterone level 3 months after the last injection was 7.5nmol/l (IQR 5.5-12.8- available in 13/15 patients - two stopping treatment early). By 6-9 months after the last injection, the testosterone level was 9.7nmol/l (IQR 5.7-11.1). Mean testicular volume 6-9 months after last injection was 6.9ml (1.9), significantly greater than the baseline 2.2mls (SD 0.2; $p<0.001$). In keeping with this, LH and FSH levels also remained significantly raised compared to baseline at 5.3IU/l (IQR 3.2-5.9, $p<0.001$) and 7.9IU/L (IQR 5.1-10.4, $p<0.001$) respectively.

Muscle function

The mean NSAA score at baseline for ambulant participants was 13.7 (SD 9.8) (Table IV). The mean PUL score at baseline was 33.9 (SD 6.7) and was not directly correlated with ambulatory status (Table I). PUL score was 30.9 (SD 8.7) at final assessment which was no different to baseline. Five participants lost ambulation during the 2-year study period, so that only 8 completed the NSAA at the final visit. For those with initial and final NSAA scores, mean initial NSAA score was 18.0 (SD 8.9, range 6-30) and final NSAA score 13.0 (SD 7.2, range 4-24).

Anthropometric variables

Baseline height z-score was -3.17 (SD 1.34) and -3.44 (SD 1.06) after 2 years. The mean 2-year height gain was 8.8 cm (SD 5.2). The baseline BMI z-score was 2.16 (SD 0.90) with a mean change of -0.45 (SD -3.2) over 2 years, giving a final BMI z-score of 1.64 (SD 1.35). There was no change in fat mass or lean mass index during the study (Table IV, Figure III).

Bone mineral density

Initial lumbar spine size adjusted z-score was 0.22 (SD 2.21). After 2 years of testosterone treatment, the lumbar spine size-adjusted z-score was 0.35 (SD 2.21).

Bone age

Bone age advanced in all patients during the study period (Tables I and II); mean change was 3.0 years (SD 1.7).

Muscle MRI

Cross-sectional comparison of DMD participants vs controls

Data were obtained from the legs of 5 healthy controls and the arms of 6 healthy controls. Arm and leg muscle MRI data was obtained for 14 DMD participants who attended at baseline, 1 year and 2 years.

For leg muscle groups, the muscle FF was higher in DMD participants than controls (p = <0.001, Table VI), with a wide variation in leg muscle pathology of individuals (Figure IVA). The tibial bone marrow FF of DMD boys was also higher than controls at baseline (99.3% vs 88.9%, p = 0.02, Table VI).

All arm muscles had higher FF for DMD participants than controls (p <0.01, Table VII). The extent of arm muscle involvement varied considerably between individuals (Figure IVB). T2 relaxation times were higher in DMD patients at baseline (p values <0.01) than controls (Table VII). The difference between T2 times of participants and controls at baseline was least marked for the UFG (1.3ms vs 2.6-3.0ms, Table VII).

Longitudinal changes in MRI data

The bone marrow FF was constant over time (Table VI). There were no significant longitudinal changes in mean FF of any individual leg muscle (Table VI), and when leg muscle groups were combined, there were no significant changes in FF during the 2-year period. When all muscle groups were combined, there were significant increases in both CSA and cCSA between baseline and 2 years (p<0.001 and p<0.01 respectively). When CSA of combined muscle groups for individuals was analysed, 8 participants had a significant change in CSA over 2-years. By individual muscle group, cCSA only increased in TA.

Longitudinal changes in MRI arm data

There was a significant increase in FF of the UFG (Table VII) over the 2-year period. Arm FF remained unchanged in most participants/muscle groups over the 2-year period (Table VII and Figure IVB). When all arm muscle groups were combined, both CSA and cCSA increased (p=0.004 and p=0.05). The CSA was greater in all four muscle groups at 2 years (Table VII).. The only significant change in arm cCSA over time was in the FEG, with increases at 1 and 2 years compared to baseline (Table VII).

The T2 relaxation times for all groups except UFG decreased significantly at 2 years post-baseline but remained higher than controls (Table VII).

Discussion

The newly revised standards of care highlight the importance of the timely recognition and management of pubertal delay in DMD (19) and this is the first study that has prospectively evaluated pubertal induction in adolescents with DMD. Our data support the use of androgen in DMD boys on GC with evidence of benefit in many of the parameters studied. Monthly incremental injections of Sustanon were well tolerated with adolescents being mainly 'very satisfied'. The participant who awarded a value of 1 to the question, "Is the treatment easy to use?" probably did so in error, as they awarded the maximum score of 7 to the other questions. The only domain in which the score was lower was 'time to work' where participants were 'satisfied'.

All patients were in late puberty after 2 years. The increase in gonadotropin levels and testicular volume, which occurred by 3 months and persisted at 6-9 months after the last Sustanon injection, suggested activation of the hypothalamo-pituitary gonadal (HPG) axis and endogenous testosterone production after the cessation of exogenous testosterone. There was variability in terms of testicular volume, androgen and gonadotrophin levels, suggesting that there may be additional factors affecting the HPG axis including relative sensitivity to GC (20, 21). It will be important to determine their ongoing gonadal and androgen status.

There was a mean height increase of 8cm over 2 years, substantially more than the median height velocity of only 0.45cm/year in the year preceding pubertal induction described previously in DMD (8). Short stature is a key concern in this population (6), and many adolescents would like to be taller and look more like their peers. Whilst a large height gain may not be advantageous (22), a small height gain during puberty is likely to be welcomed by the patients.

Delayed bone age in adolescents with DMD is multi-factorial, but largely related to delayed puberty and growth retardation (23, 24). Bone age increased at a greater rate than chronological age suggesting that epiphyseal maturation was taking place despite the presence of pharmacological doses of GC.

Low BMD and fracture are well-recognised complications in DMD, both as a consequence of the disease process itself and chronic GC use. Although this study was not powered to look in detail at bone health or body composition, it appears that the testosterone regimen stabilised BMD. However, as shown in Figure III, there was wide variability, with some participants having a marked increase in BMD, whilst others remained stable or decreased. Studies have shown that BMD decreases with age and GC use in DMD and falls further still when boys lose ambulation (25, 26, 27, 28). The process of puberty is vital for an increase in bone size and bone mineral content. Androgen deficiency is a risk factor for osteoporosis and fracture and early initiation of androgen therapy is associated with improved bone mineral density (29). Although fracture risk does not correlate directly with BMD, these data suggest a possible bone protective effect of testosterone therapy, despite ongoing GC use. Many boys lose ambulation after they fall and fracture and if muscle mass and bone density

246 can be optimised during puberty, ambulation may be maintained for longer. The change in
247 mean PUL score over two years is consistent with published data (-3 points), (30) whereas
248 the fall in mean NSAA score was slightly less than reported in the literature - mean drop of 4
249 points a year (31).
250
251 When arm muscle groups were combined, there were significant increases in both CSA and
252 cCSA from baseline to 2 years. Only one participant (participant 7, non-ambulant at baseline)
253 had a significant increase in overall FF of the arm. The arm FF remained unchanged over the
254 2-year period, in contrast to the natural history of DMD. Our data contrast with a recent
255 study of 15 non-ambulant boys with DMD (mean age 13.3 years) which found a progressive
256 increase in FF (18). Only 7 boys completed that study and pubertal status was not recorded.
257
258 When leg muscle groups were combined and compared between baseline and 2 years, there
259 were also increases in both CSA and cCSA. FF in most participants and muscle groups were
260 stable over the 2-year study period and only increased in one participant. This challenges
261 the results found in other studies (32, 33).
262
263 The CSA of all four muscle groups in the arm increased significantly over 2 years, showing
264 the muscle growth that we would expect. The cCSA (that area which is not fat replaced) on
265 the other hand only increased significantly for the FEG, suggesting that in this muscle group
266 the growth of muscle volume outpaced the disease process, whereby fat replaces muscle
267 and the FF results support this. When all muscle groups were considered together, there was
268 no significant increase in fat fraction and significant increases in CSA and cCSA for arm and
269 legs: muscle growth at least kept pace with chronic destruction. By contrast, the cCSA did

not significantly increase in any of the arm muscle groups studied by Ricotti et al (18).

The mean FF of the tibial bone marrow for the DMD boys was significantly higher than controls (Table III). Many factors may contribute to BMD reduction including progressive muscle weakness and GC use, which cause increased bone marrow adiposity (34, 35), probably through a diversion in the mesenchymal progenitor cell line with increased adipogenesis (36, 37). Bone marrow FF has been suggested as a potential biomarker in postmenopausal women with fragility fractures (38).

The T2 relaxation times of all four compound muscle groups were significantly higher at baseline than controls, in keeping with an ongoing inflammatory process. This observation is consistent with some studies (39) but contrasts with others (32). The relaxation times for all groups except UFG (which was the least affected at baseline) decreased significantly at 2 years but remained higher than those of the controls at baseline. This may indicate a reduction in the inflammatory process after testosterone supplementation, as similar results have been demonstrated after 3 months of GC therapy in DMD (40). Androgens are known to have an anti-inflammatory effect, partly via their role in cytokine-suppression (41, 42) and may have a disease-ameliorating role in addition to the more well characterised benefits of testosterone during puberty. It is possible, however, that T2 relaxation time may decrease once a boy loses ambulation and 5 boys lost ambulation over 2 years.(32).

Limitations of the study

Although we wanted to perform a randomised double-blinded clinical trial, the Research Ethic Committee felt that it would be inappropriate to withhold treatment for a period of 2

years in this cohort, particularly as our audit suggested that it was well-liked (8). Furthermore, physical development during puberty precludes the use of a placebo. By standardising the regimen, investigations and collection of the adverse event profile and safety data that are required as part of a rigorous clinical trial methodology, this has enabled us to systematically evaluate the tolerability and efficacy of this regimen. The study was small, and the recruitment target was pragmatic within a 1 year window at our centre. It was therefore underpowered to detect differences in some of the secondary outcomes such as BMAD and body composition.

Conclusion

A 2-year incremental regimen of 4-weekly intramuscular testosterone injections in pre-pubertal boys with DMD was safe, well tolerated, associated with high satisfaction scores and resulted in endogenous gonadotropin release when exogenous testosterone was stopped. Height increased in most individuals and there was evidence to suggest a favourable impact on bone density, muscle morphology and muscle function. Our data support the routine use of exogenous androgen in boys with DMD who are on GC, as recommended in the revised DMD standards of care. It is unclear whether endogenous testosterone production will remain adequate in the longer term.

Declaration of Interest

Volker Straub has received speaker honoraria from Sanofi Genzyme and is or has recently been on advisory boards for Audentes Therapeutics, AveXis, Biogen, Exonics Therapeutics/Vertex, Roche, Sarepta Therapeutics and Wave Therapeutics. He has research collaborations with Ultragenyx and Sanofi Genzyme.

Robert Muni Lofra has received speaker honoraria from Biogen and has been recently on advisory boards for Roche and Biogen.

Anna Mayhew has participated in SAB meetings for Summit, PTC and Biogen and performs Consultancy work (training physiotherapists) for: Roche, Pfizer, PTC, Summit, Sarepta, Santhera, Italfarmaco, Amicus, Biogen and Avexis.

Michela Guglieri has received speaker honoraria from Sarepta and is or has been on advisory boards for PTC Therapeutics and Pfizer.

Claire Wood, Kieren Hollingsworth, Eric Hughes, Sadha Punniyakodi, Rod Mitchell and Tim Cheetham have no conflicts of interest.

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488

	Overall	Ambulant (n=12)	Non-ambulant (n=3)
		Mean (SD)	
Age at baseline (years)	13.8 (1.50)	13.7 (1.42)	14.4 (1.99)
Bone age at baseline (years)	9.4 (2.2)	9.1 (2.2)	10.5 (2.3)
Time on steroids (years)	8.4 (2.2)	8.4 (1.2)	8.7 (4.9)
Deflazacort dose (mg/kg) n=9	0.55 (0.13)	0.56 (0.14)	0.44 (-)
Prednisolone dose (mg/kg) n=6	0.47 (0.13)	0.51 (0.15)	0.41 (0.11)
North Star Ambulatory Assessment score (NSAA)	13.7 (9.8)	13.7 (9.8)	-
Performance of the upper limb (PUL) score	33.9 (6.7)	34.3 (7.1)	32.7 (6.1)

489 **Table I** Baseline characteristics of participants

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	Initial age (years)	Initial bone age (years)	Final age (years)	Final bone age (years)	Change in bone age (years)	GC regimen	GC daily dose (mg/kg)	Initial NSAA	Final NSAA	Initial PUL	Final PUL
1	14.75	8	16.75	12.5	4.5	D daily	0.52	7	8	36	31
2	13.33	8	15.33	11	3	P daily	0.67	4	NA	26	17
4	14.75	11	16.75	12.5	1.5	P daily	0.59	11	NA	41	34
5	12.42	12.5	14.42	14	1.5	D daily	0.44	NA	NA	34	39
6	14.50	8	16.50	11	3	P daily	0.33	NA	NA	38	29
7	16.33	11	18.33	12.5	1.5	P daily	0.48	NA	NA	26	20
8	12.08	6	14.08	8	2	D daily	0.5	16	17	40	41
9	12.33	10	14.33	12.5	2.5	D daily	0.49	30	24	42	42
10	16.83	12	18.83	12.5	0.5	D daily	0.69	4	NA	20	22
11	12.83	9.5	14.83	14.5	5	P 10 on/off	0.38	23	21	41	38
12	14.17	6	16.17	11.5	5.5	D daily	0.69	13	4	28	22
13	14.83	10	16.83	13.5	3.5	P daily	0.38	23	14	40	41
14	12.25	12.5	14.25	14	1.5	D daily	0.3	1	NA	31	21
15*	13.08	9	15.08	13	4	D daily	0.62	26	10	31	36
16*	13.08	7	15.08	13	6	D daily	0.69	6	6	35	31

Table II Individual patient characteristics D: deflazacort, P: prednisolone, NSAA: North Star ambulatory assessment, PUL: performance of the upper limb. * signifies the 2 patients that chose to stop testosterone treatment after 18 months

	Q1 (1-7)	Q2(1-7)	Q3 (1-7)	Q4 (yes/no)	Q5 (1-5)	Q6 (1-5)	Q7 (1-5)	Q8 (1-5)	Q9 (1-7)	Q10 (1-7)	Q11 (1-7)	Q12 (1-5)	Q13 (1-5)	Q14 (1-7)
	Effectiveness			**Side-effects**					**Convenience**			**Global satisfaction**		
1	7	7	6	No					6	6	5	5	5	7
2	7	7	5	No					6	6	6	4	No data	7
4	7	7	7	No					7	5	5	4	3	6
5	6	6	6	Yes	2	1	1	1	6	6	2	4	5	7
6	7	7	7	No					7	7	7	5	5	7
7	5	4	5	No					6	5	5	3	3	5
8	4	3	5	No					6	6	7	4	3	5
9	7	7	7	No					7	7	6	5	5	7
10	6	4	5	No					7	7	7	3	3	5
11	6	6	6	No					6	6	6	4	3	6
12	6	6	6	Yes	1	1	1	1	6	6	5	5	5	6
13	6	6	5	No					6	5	5	4	4	7
14	5	5	5	No					7	7	6	5	5	7
15*	4	7	5	No					5	5	5	3	2	5
16*	4	4	2	No					1	7	7	3	3	7

Table III TSQM questionnaire data. Q4 is a dichotomous response option with a conditional skip to Q9. Where a scale of 1-7 was used, 1 indicates 'extremely dissatisfied', 7 indicates 'extremely satisfied'. Within the scale of 1-5, 1 indicates 'not at all' while 5 indicates 'extremely'.

	Initial T	Initial LH	Initial FSH	Initial testic vol (mean)	Initial Tanner stage	T at end of regimen	LH at end of regimen	FSH at end of regimen	Testic vol at end	Tanner stage at end	T 3 m after	LH 3 m after	FSH 3m after	T 6-9m after	LH 6-9 m after	FSH 6-9 m after	Testic vol 6-9m after
1	0.1	<0.1	1.1	1.5	G1P1	10	0.1	1.4	1.5	G5P4	15.2	8.8	3.6	14.7	3.2	5.5	4.75
2	0.1	<0.1	1.3	2	G1P2	18.7	0.1	0.1	2.5	G4P4	21.8	5	1.8	11.1	3.6	2.5	10
4	1.9	3.3	3.8	3.5	G2P1	10.3	4.9	13.1	3	G4P4	19.5	6.2	11.2	9.7	3.2	11.1	8
5	0.1	<0.1	0.5	1.5	G1P1	8.3	0.1	0.1	3	G4P4	7.5	9.8	4.4	5.7	2.6	4	8
6	0.1	1.6	7.7	3	G1P2	17.9	0.1	0.1	4	G4P4	12.1	25.1	38.3	11.7	13.7	32.9	5
7	0.1	<0.1	1.8	3.75	G1P1	5.2	0.5	0.1	3	G4P5	6.6	5.9	13.4	5.1	8.4	12.6	8
8	0.1	<0.1	1.2	1.5	G1P2	20.7	0.1	0.1	3	G5P5	8.7	6.7	6.7	9.7	5.9	6.5	4
9	0.1	<0.1	<0.1	2	G1P1	5.7	0.1	1.2	2	G5P4	5.5	7.6	8	7.9	5.3	8.6	10
10	0.1	<0.1	<0.1	1.5	G1P2	6.9	0.1	0.1	2.5	G5P4	1.9	0.1	3.9	3.8	13.5	9.1	4
11	0.1	<0.1	<0.1	2	G1P1	6.8	0.1	0.1	3	G5P5	6.7	6	6	10.1	5.5	8.4	-
12	0.1	<0.1	1.1	2	G1P1	8.9	0.1	0.1	5	G5P5	11.7	7.8	5.9	9.8	4.2	5.1	5
13	0.1	<0.1	1.1	2	G2P2	7.4	0.1	0.7	2	G4P4	6.9	10.1	3.6	8.3	3.1	2.0	-
14	0.1	<0.1	1.3	1.5	G1P1	31.4	0.1	0.1	2.75	G5P5	1.3	2.3	1.8	4.9	5.7	10.4	-
15*	0.1	<0.1	3.9	3.25	G2P1	4	0.1	0.1	2	G4P4	-	-	-	4.8	2.9	7.8	7.5
16*	0.1	<0.1	1.1	2	G1P2	5.2	6.4	7.2	3	G5P5	-	-	-	12.8	5.3	7.9	6

Table IV Pubertal characteristics * signifies the 2 patients that chose to stop testosterone treatment after 18 months. T measured in nmol/l, FSH and LH in IU/L, testicular volume in mls (mean of left and right volumes).

	Before Testosterone	After Testosterone
	Mean (SD)	
Height (cm)	133.6 (10.7)	142.4 (7.4)
Height z-score	-3.17 (1.34)	-3.44 (1.06)
Target height (cm)	183.3 (4.6)	
Weight (kg)	48.3 (12.4)	52.8 (12.2)
Weight z-score	-0.19 (1.56)	-0.94 (1.81)
BMI (kg/m^2)	26.8 (4.5)	26.3 (5.0)
BMI z-score	2.16 (0.9)	1.64 (1.35)
Lean mass index (kg/m^2)	12.4 (2.3)	12.8 (2.9)
Fat mass index (kg/m^2)	13.6 (4.2)	12.5 (4.3)
NSAA (if ambulant at both timepoints)	18 (8.88)	13 (7.23)
PUL	33.9 (6.72)	30.9 (8.66)
LS BMAC z-score	0.22 (2.21)	0.35 (2.21)

Table V Outcome variables before and after a 2-year incremental regimen of intramuscular testosterone.

Muscle group	DMD bl FF,%	DMD 1y FF,%	DMD 2y FF,%	Control FF,%	DMD bl CSA,mm^2	DMD 2y, CSA,mm^2	DMD bl, cCSA,mm^2	DMD 2y, cCSA,mm^2
BM	99.3	99.3	99.2	88.9*	-	-	-	-
TA	11.7	11.2	11.9	2.0†	296	383†	265	343†
MG	23.6	22.7	22.6	2.8§	1216	1374	942	1090
LG	28.7	28.0	28.5	2.5§	549	644	371	443
SOL	19.1	20.1	21.5	2.4‡	1566	1611	1261	1274
RF	51.9	53.9	51.5	1.6§	500	548	237	258
VL	50.8	51.5	51.8	2.0§	994	1069	489	515
BFLH	59.5	61.4	62.0	2.7§	839	856	316	335
ST	28.2	27.8	32.7	2.7‡	508	612†	375	425
SART	24.9	26.1	27.5	5.1§	215	244*	163	178
TOTAL	**33.9**	**34.1**	**33.8**	**2.4‡**	**6683**	**7341‡**	**4419**	**4861†**

Table VI Longitudinal results for fat fraction (FF), cross-sectional area (CSA) and contractile cross-sectional area (cCSA) when each muscle group is considered separately for 14 participants.* $p < 0.05$, † $p < 0.01$, ‡ $p<0.001$, § $p < 0.0001$ – p values in the control column are unpaired t-tests vs DMD baseline, p values in DMD 1 y and 2y are paired tests vs DMD baseline. Abbreviations : BM: bone marrow, TA: tibialis anterior, MG: medial gastrocnemius, LG: lateral gastrocnemius, SOL: soleus, RF: rectus femoris, VL: vastus lateralis, BFLH: biceps femoris long head, ST: semitendinosus, SART: sartorius.

Muscle group	DMD bl T2,ms	DMD 1y T2,ms	DMD 2y T2,ms	Control T2,ms	DMD bl FF,%	DMD 1y FF,%	DMD 2y FF,%	Control FF,%	DMD bl CSA,mm^2	DMD 2y, CSA,mm^2	DMD bl, cCSA,mm^2	DMD 2y, cCSA,mm^2
FEG *(n=12)*	31.0	31.0	29.5‡	28.3§	11.5	10.3	11.5	3.9‡	397	501*	354	450*
UEG *(n=12)*	32.4	32.1	31.2†	29.5‡	13.6	16.1	18.8	3.4†	865	960*	749	808
FFG *(n=12)*	31.5	31.2	29.6†	28.5§	13.2	12.8	13.3	3.7†	849	911*	762	815
UFG *(n=13)*	30.4	30.2	30.2	29.1*	17.0	21.2	24.8*	3.6†	564	630*	480	490
TOTAL					**12.3**	**11.8**	**14.6**	**3.6***	**2675**	**3002†**	**2345**	**2563***

Table VII Longitudinal results for fat fraction (FF, %), mean T2 relaxation time (ms), cross-sectional area (CSA, mm^2) and contractile cross-sectional area (cCSA, mm^2), when each muscle group is considered separately for the participants. * $p < 0.05$, † $p < 0.01$, ‡ $p<0.001$, § $p < 0.0001$ – p values in the control column are unpaired t-tests vs DMD baseline, p values in DMD 1 y and 2y are paired tests vs DMD baseline. Abbreviations: FEG: forearm extensor group, UEG: upper arm extensor group, FFG: forearm flexor group, UFG: upper arm flexor group